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ST CYRES LECTURE

Endothelium in control

A H Henderson

Clinical science has evolved since the days of Sir Thomas Lewis, who gave the St Cyres lecture in 1931 and whose eponymous chair I occupy. It involves still the penetration of astute clinical observation, but has come increasingly to embrace the elucidation of underlying mechanisms in the more controlled conditions of the laboratory. Its compass is being stretched by the reductionism of molecular and cell biology, but these exciting developments do not exonerate us from the ever daunting task of seeking to understand the coordinated behaviour of the whole. Perhaps biomathematics will give new impetus to our efforts to discern form in the noise. Never was there greater need for cross-talk between the different scientific disciplines and between scientists and clinicians.

The greatest growth area in cardiovascular science over recent years must surely be in the role of endothelium. Not only is there a lot of it—equivalent in mass to five normal hearts and in area to half a dozen tennis courts per standard 70 kg man—but it is coming to be recognised as a cardiovascular endocrine organ in its own right, occupying a critically strategic interface between blood and body, and subserving a multitude of regulatory roles. These range from acting as a selective permeability barrier, through vasomotor control, pro- and antithrombotic mechanisms and regulation of vascular growth, to metabolic and immunological activity. We here consider just one—namely the production of endothelium derived relaxing factor (EDRF), a powerful vasodilator substance released from the endothelium of all blood vessels of all species studied.

Endothelium derived relaxing factor

THE PHENOMENON DISCOVERED

Before 1980, the existence of EDRF was unknown. It was in 1980 that Furchgott and Zawadski published their now classic paper describing endothelium dependent vasodilatation to acetylcholine.[1] They had elucidated the paradox, long known to pharmacologists, that acetylcholine was vasodilator in vivo yet vasoconstrictor when studied in vitro with arterial strip preparations (where it transpired that the delicate endothelium is generally inadvertently removed during preparation). This explained the findings of our own studies at that time, for we had serendipitously (and initially unknowingly) encountered the phenomenon while developing an isolated perfused coronary artery preparation of the rabbit to study vasomotor regulatory mechanisms. As we gained experience with the preparation, frustratingly we met with increasing difficulty in getting the arteries to constrict.[2] Indeed constrictor responses to the usual vasoconstrictor agents were virtually abolished with better preparation due, it became apparent, to endothelial preservation.

Furchgott had suggested that the endothelium dependent vasodilator influence might be a humoral factor.[1] We developed a cascade bioassay system in which effluent from a perfused endothelialised "donor" artery perfused a denuded, pre-constricted "recipient" artery,[3] and were able to confirm that the phenomenon was indeed due to endothelial production of a humoral agent, EDRF, both tonically in the basal state and to a greater extent when stimulated, for example, by acetylcholine. By experiments in which we altered the transit time between donor and recipient vessels, we showed that EDRF is unstable, with a half life measured in seconds,[3] though it is likely to be less than a second in vivo.[4]

EDRF: THE ENDOGENOUS NITROVASODILATOR

The possibility that EDRF might be nitric oxide emerged.[5–7] Direct evidence for this was provided by Palmer and colleagues in 1987.[8] There has, however, remained a suspicion that EDRF may be not nitric oxide itself but a closely related molecule.[9] EDRF is thus the endogenous counterpart of the nitrovasodilator drugs.[10] The metabolic step involved in the production of EDRF is that of activating nitric oxide synthase to provide nitric oxide from its precursor substrate, L-arginine,[11] the supply of which seems not normally to be rate-limiting because it can be regenerated endogenously from other amino acids. The short half life of EDRF is thus explained, for nitric oxide is rapidly converted to nitrite and nitrate in the presence of water and oxygen, and even more rapidly by superoxide radicals, which are widely present in biological systems.[12 13]

ERDF RELEASE

The list of conditions now known to stimulate EDRF release is large (for reviews, see[14–17]). It includes agents liberated during platelet aggregation and thrombosis (serotonin, ATP and ADP, thrombin) (thereby implying a further mechanism whereby healthy endothelium inhibits thrombosis) and a large number of hormones and neurotransmitter substances (for example, substance P, calcitonin gene

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related peptide, acetylcholine, noradrenaline, vasopressin, vasoactive intestinal peptide, bradykinin, histamine) (implying means whereby (*a*) intravascular agents may transduce their signals across the endothelial barrier and (*b*) agents liberated from adventitial autonomic nerve endings on smaller arteries may exert their action after diffusion through to the endothelium). As an experimental tool, calcium ionophore can be used to stimulate EDRF release independently of receptors. Perhaps the most important physiological stimulant of EDRF release, though, is flow rate[18] acting through the relatively small longitudinal shear force experienced uniquely by the endothelium—as confirmed by experiments with fluids of different viscosity[19] and as increased with pulsatile flow.[20]

The mechanism of agonist stimulated release of EDRF from endothelial cells involves occupation of specific receptors, leading to activation of the phosphoinositol pathway and an increase in cytosolic calcium,[21-28] both from release of internally stored calcium (which causes a transient high calcium level) and from continuing influx of extracellular calcium (which maintains a level of calcium sufficient to stimulate EDRF production but which is lower that that which would stimulate prostacyclin production).[29] Shear force is thought to act through altering potassium conductance, leading by ionic interchange to an increase in cytosolic calcium.[30-32] Calcium (via calcium calmodulin) activates nitric oxide synthase[33] to produce EDRF. The stimulated production of EDRF is dependent also on the provision of mitochondrial ATP.[34] Some negative feedback control exists in that EDRF activates soluble guanylate cyclase in endothelial as in other cells (see below) and thus inhibits its own production in response to some agonists.[35 36]

ACTION OF EDRF

Nitric oxide interacts with the haem moiety present in the cytosolic enzyme, soluble guanylate cyclase, to activate it and thereby raise intracellular concentrations of cyclic GMP.[10 37-40]

An increased intracellular concentration of cyclic GMP inhibits the agonist induced activation of the phosphoinositol pathway, which is responsible for stimulating calcium influx and intracellular calcium release and thus for increasing cytosolic free calcium.[41-44] An increase in cyclic GMP in vascular smooth muscle cells therefore relaxes vascular smooth muscle tone, particularly where this is increased by receptor mediated stimulation.[45] It has analogous effects in other cell types—for example, platelets, myocardium (see below).

Haemoglobin also contains a haem moiety with which nitric oxide competitively interacts.[46 47] The sink of haemoglobulin within erythrocytes,[48] as well as haemoglobin complexed to haptoglobin in plasma,[49] ensure that EDRF has no downstream activity within the vascular compartment, as experimentally confirmed.[50] Its action is thus localised to the immediately subjacent vascular smooth muscle. Each millimetre of endothelium controls its own little bit of the vascular system.

VESSEL DIFFERENCES

EDRF activity has been demonstrated in every vessel studied—arteries, microvessels, and veins—and in every species studied,[14 16 17] with the implication that it is of primitive evolutionary origin. There are however considerable differences in the level of activity in different vessels.[2 51-54] Differential bioassay has confirmed that these are due to differences both in the response to EDRF and in its release.[55] These may be differences in basal release, flow related release, or release resulting from receptor stimulation, also with differences in specific receptor responsiveness. Differences in observed responses must also take account of differential baselines, as set by the levels of basal or flow related EDRF activity, because a "response" represents the difference between the starting level (baseline) and the end point (ceiling) of the response and will be as much influenced by the basal as by the stimulated level.[2 56] Furthermore, many of the known stimulants of EDRF act not only on endothelial receptors but also on vascular smooth muscle receptors (not always of the same subtype). The resultant response to such a "double agent" will thus depend on the relative strengths of the EDRF mediated dilator response and the direct constrictor response. Clearly there is rich potential for variation in different blood vessels, and under different physiological conditions let alone pathological ones.

It has generally been considered that veins show lower EDRF activity than arteries.[51 57] Bioassay experiments have suggested that, at least in some cases, the difference may lie more in the response to EDRF than in its release.[58] At first sight, this runs counter to the generally held clinical view that the major site of action of its pharmacological analogue, the nitrovasodilator drugs, is on the venous system.[59] This nitrovasodilator selectivity, however, probably reflects the need for most organic nitrovasodilator drugs, such as glyceryl trinitrate or the isosorbide nitrates, to undergo metabolic conversion to provide the active principle, nitric oxide, and veins seem better endowed with this metabolic pathway than arteries (while platelets seem to lack it entirely)[60 61]—these considerations do not apply to molsidomine, SIN-1, or sodium nitroprusside which are sources of nitric oxide that do not depend on this metabolic step. Comparison of EDRF activity between different blood vessels is in practice difficult because the question is not as simple as it appears. Bioassay experiments have indeed confirmed the ability of veins (for example, human saphenous vein) to relax in response to EDRF[62] and of human saphenous vein to produce EDRF.[58] However, EDRF activity depends not only on EDRF production and the response to EDRF, but also on the specific agonists used to cause constriction and to stimulate EDRF release. Much of

the apparent controversy in published reports is attributable to limitations inherent in the techniques used and to vessel specific differences between endothelial responsiveness to different agonists.

Microvessels

EDRF IN VIVO

Tonic microvascular EDRF activity has been demonstrated by infusing or feeding analogues of arginine which block nitric oxide production. In the intact rabbit, for example, this causes a substantial, prolonged but reversible increase in blood pressure—interestingly without calamitous platelet aggregation.[63] Inhibition of coronary EDRF activity limits perfusion of isolated buffer perfused hearts to the point of inducing global ischaemia.[64] In the human forearm, intra-arterial infusion of an arginine analogue likewise reduces flow, illustrating the contribution of tonic microvascular EDRF activity to the human circulation.[65]

EDRF AND FLOW IN VASCULAR NETWORKS

The influence of EDRF activity on the coordinated behaviour of an intact microvascular bed has been investigated in the perfused rabbit ear, using microradiographic techniques to image simultaneously different generations of microvessels (down to about 100 μm diameter) and observe their calibre in response to changes in flow in the presence and absence of EDRF activity.[66-69] These studies illustrate the interdependence of different vessels within the bed and emphasise the need to consider the integrated behaviour of the whole vascular bed. For example, a pharmacologically induced increase in distal resistance can raise intravascular pressure and lead paradoxically to proximal dilatation, while a reduction of resistance in one part of a bed can lead to EDRF mediated dilatation as a result of increased flow throughout the bed.

Flow related EDRF activity amplifies a locally induced change in resistance in the bed, thus contributing, for example, to a metabolically mediated hyperaemic response. It also coordinates the changes in calibre throughout the bed. Furthermore, it reduces the increase in pressure needed to drive increased flow: specifically, flow (Q) was shown to be related to diameter (D) to the fourth power ($Q = aD^4 + b$, where a and b are constants) when EDRF was present but not in its absence, implying that EDRF results in progressively reduced increments of pressure in order to increase flow at high flow rates. Moreover, EDRF activity was necessary to preserve constancy of flow distribution at different flow rates: in the absence of EDRF activity, flow distribution became heterogeneous—a form of "steal".

BRANCHING GEOMETRY OF VASCULAR BEDS

Analyses of vessel diameters in relation to flow in the rabbit ear preparation have shown that the pattern of branching angles in the bed provides for optimal minimisation of power losses—an optimality of design which is lost in the absence of EDRF activity.[70]

AUTOREGULATION

The "myogenic response", which is intrinsic to vascular smooth muscle in most beds, describes the constriction induced directly by an increase in intraluminal pressure. This is a positive feedback mechanism which makes for a potentially unstable situation unless balanced by an opposing positive feedback mechanism. EDRF may be seen as answering this need, for pressure is normally coupled to flow and increased flow leads to EDRF mediated vasodilatation, thus providing a positive feedback mechanism in the opposite direction.

The myogenic response is predominantly responsible for autoregulation of flow, whereby the flow is maintained relatively constant despite changing pressure within limits. EDRF opposes this phenomenon. The relative strength of these two mechanisms determines the degree of autoregulation, as appropriate to the biological needs of different beds.[71]

PHYSIOLOGICAL IMPLICATIONS

EDRF activity is high in the microvessels. In the intact network of the rabbit ear, it was particularly high in those vessels best placed to control distribution of flow (about 100–200 μm diameter) where calculated shear force was also highest. EDRF seems to have an important physiological role in maintaining "efficiency" of perfusion. Even minor impairment of EDRF activity would have adverse effects on the efficiency and work of perfusion.

Platelets

EDRF also increases cyclic GMP concentration in platelets.[72-75] This inhibits both platelet adhesion and platelet aggregation,[76] whereas agents that increase cyclic AMP (prostacyclin, adenosine) inhibit only aggregation. It is during adhesion that platelet derived growth factor (PDGF) is released. Cyclic GMP and cyclic AMP act at different sites within the cell and their effect on aggregation is synergistic.[76]

Activated platelets release agents (for example, serotonin, adenosine diphosphate (ADP)) which stimulate intact endothelium to release EDRF; EDRF inhibits further platelet aggregation and causes local vasodilatation—a negative feedback. In the absence of endothelium, the direct action of these same agents causes further aggregation and local vasoconstriction—a positive feedback. Aggregation and constriction will thus be localised to the site of endothelial damage.

Platelets seem to have nitric oxide synthase themselves.[77] Platelet activation is associated with nitric oxide production, and nitric oxide will activate soluble guanylate cyclase in the platelets to provide some negative feedback to the activation process.[77]

Endocardium

Endothelium also lines the much trabeculated cavity of the cardiac chambers. It has recently

been shown that just as vascular endothelium influences vascular smooth muscle tone so endocardial endothelium can influence contraction of underlying cardiac muscle.[78-82] Selective removal of endocardium from isolated papillary muscle preparations results in a "negative inotropic" effect which is unusual in that the duration of contraction is abbreviated but contractile behaviour early during the course of a contraction is unaltered. Effluent from cultured endocardial cells reverses this effect, thereby confirming that endocardium tonically releases a myocardial contraction prolonging factor ("endocardin"?)[80-82] of as yet unknown identity. It does not seem to be any of the obvious candidates such as an endothelin or a prostaglandin. Preliminary experiments suggest that the tonic contraction-prolonging effect of endocardium which has been shown in isolated preparations is manifest also in the intact ventricle despite the small mass of endocardium relative to myocardium.[83] Clearly, this could be important in modulating diastolic filling. Conversely, endocardium can also be stimulated to release EDRF which increases cyclic GMP concentrations[81 82 84 85] in myocardium and, like other interventions which raise myocardial cyclic GMP, shortens the duration of contraction—an effect which is indistinguishable from that of *removing* endocardium (which does not itself alter cyclic GMP concentrations).

Other sources of nitric oxide

It is becoming evident that nitric oxide is an intercellular signal which fulfils a very wide variety of physiological roles. Many cell types other than endothelium also produce nitric oxide—platelets (see above), brain,[86 87] adrenal cells,[88] non-adrenergic non-cholinergic nerve fibres,[89 90] neutrophils, monocytes,[91] and mast cells[92]; while macrophages also produce nitric oxide as part of their immunological response but probably by a different mechanism.[93 94]

Pathophysiology

There is a growing list of conditions in which EDRF activity seems to be impaired—including subarachnoid haemorrhage,[95-98] endothelial damage and repair,[99-102] ischaemia and reperfusion, atheroma, hypertension, diabetes, heart failure, lack of oestrogens,[103] and aging.[104 105] Conversely, increased EDRF production during endotoxin shock may be responsible for hypotension[106] but also perhaps for survival. Endothelium, indeed, seems to be a prime target for "cardiovascular risk factors".

SUBARACHNOID HAEMORRHAGE

Subarachnoid haemorrhage is known to be complicated by cerebral vasoconstriction. Given that haemoglobin interacts with EDRF where it can get at it, an obvious potential mechanism is suggested. Several studies now provide convincing evidence that the constriction is indeed attributable to inhibition of EDRF activity by haemoglobin.[95-98] Experimental in vivo injection of haemoglobin or whole blood into the subarachnoid space causes constriction of the intrathecal cerebral arteries associated with a reduction in their cyclic GMP content (T M Griffith, unpublished observations). The effects persist for up to a week, with histological evidence of haemoglobin in the intimal layers. This probably reflects seepage through these intrathecal arteries, which are peculiar in that they have no vasa vasorum and may be more porous than other arteries.

ENDOTHELIAL REGENERATION

Several groups have studied the effects of balloon denudation of coronary arteries followed by regrowth. Endothelium regrows within about a week but interestingly this may be followed over subsequent months by progressive and selective impairment of receptor mediated EDRF responsiveness.[99-102] These studies have important implications. Endothelial cells normally live for more than 10 years. They can obviously be kicked into rapid reproductive activity, however, by the crude insult of physical damage, after which they appear morphologically different[99 102] and can exhibit altered function for up to six months.[101] The selective impairment of EDRF responsiveness has been analysed further in the pig model, where the pattern of the impaired relaxation of coronary artery rings was shown to correspond to pertussis toxin sensitivity of these agonists and thus to dependence on a particular G protein that couples receptor occupation to cell signalling.[101 107] The endothelium seems to undergo an alteration of phenotypic expression, analogous to that which occurs in vascular smooth muscle where a change from normal contractile to synthetic phenotype underlies the proliferative growth intrinsic to atherogenesis and to the intimal hyperplasia seen after angioplasty and in graft stenosis.

ISCHAEMIA AND REPERFUSION

Ischaemia followed by reperfusion results in specific and probably prolonged impairment of EDRF responsiveness while endothelium independent responses remain unaltered.[108-111] EDRF activity is not impaired after ischaemia alone but becomes impaired progressively during the early minutes of reperfusion. Most studies have used rather long periods (for example, 60 min) of ischaemia,[108 109] but impaired EDRF responses in conduit coronary arteries have been demonstrated after even 15 minutes in vivo ischaemia (which also alters microvascular endothelial function as evidenced by protein leak).[112] Similar specific impairment of EDRF activity is evident in the coronary conduit and resistance vessels in the intact heart. The changes are probably secondary to mediators derived from reperfused ischaemic myocardium rather than reoxygenation of ischaemic endothelium itself, for endothelial cells in culture are remarkably resistant. Microvascular polymorph adhesion and plugging are known to occur during ischaemia and reperfusion.[113] Neutrophil activation releases superoxide radicals and impairs EDRF activity.[114 115] If adhesion is prevented by

specific antibodies, this sequence of events is prevented.[116] Lymph draining ischaemic myocardium has been shown to contain agents chemotactic for polymorphs.[117] Adenosine which increases neutrophil cyclic AMP content and inhibits superoxide production,[118] can on the other hand ameliorate polymorph plugging and improve reflow.[109] Conversely, intracoronary infusion of complement components can cause transient polymorph adhesion and plugging even in the absence of ischaemia.[119] The suspicion is thus that oxygen free radicals are involved in this impairment of EDRF production and that they are derived predominantly from activated neutrophils in vivo.

Selective impairment of endothelium dependent relaxation evoked in vitro by aggregating platelets has been demonstrated for up to 12 weeks after reperfusion.[110] Another consequence of impaired microvascular EDRF activity after reperfusion might be a redistribution of perfusion at the expense of the vulnerable endocardium.[111]

ATHEROMA

A wide range of hyperlipidaemic atheroma models, ranging from the rabbit to the primate and from hereditary to dietary hyperlipidaemia, have consistently shown impairment of receptor mediated EDRF responsiveness in large (for example, coronary) arteries while endothelium independent dilator and constrictor responses are preserved.[120–127] It was at first thought that the layer of lipid deposition acted as a barrier interfering with EDRF diffusion to the underlying vascular smooth muscle, but it is now known that endothelial production of EDRF is impaired. The phenomenon is reversible though this takes as long as 18 months in the primate model[123] and the abnormalities have persisted for 10 weeks in the rabbit model.

Does an insult to the endothelium also affect the less easily studied microvessels? As might have been predicted, abnormalities have now been reported also in the microvessels, specifically in respect of EDRF responsiveness to acetylcholine, bradykinin, and the calcium ionophore in coronary microvessels (100–200 μm in diameter) whereas the endothelium independent response to adenosine and nitroprusside remained normal.[128 129] The experimental hyperlipidaemia that is responsible for inducing atheroma—with intimal hyperplasia, "foam" cells, and fibrosis—in the large arteries clearly also impairs endothelial function in the microvessels, with potentially adverse consequences for the "efficiency" and homogeneity of flow distribution (see the section on coronary artery disease, below).

Conversely, feeding with fish oil may enhance EDRF activity.[126 130–133] We may expect to hear more of dietary manipulation of the lipid content of cell membranes and their influence on properties such as endothelial responsiveness to stimulants of EDRF activity.

CORONARY ARTERY DISEASE

Early experimental studies with coronary artery preparations where intact endothelium abolished conventional vasoconstrictor responses had illustrated vividly how localised damage provided a model of non-specific localised constriction or "spasm".[2] Studies with clinical coronary artery disease have now produced an impressively consistent body of evidence showing that specific EDRF responsiveness is impaired in the large coronary arteries, as measured by angiographic calibre of these arteries in response to a number of interventions. Dose responses to the "double agent", acetylcholine, show a shift from dilatation in normal arteries towards increasing degrees of constriction in relation to graded severity of underlying atheroma, as evidenced by "irregularity" or "stenosis".[134 135] A similar shift towards constrictor responses was observed in angiographically smooth arteries in relation to the presence of overt disease in other coronary arteries,[136] or to the presence of known risk factors for atheroma,[137] or indeed to age.[105] The inference is that the EDRF mediated dilator response to acetylcholine is depressed in the presence of atheroma, of which angiographic evidence is an insensitive measure given the remodelling which preserves vessel calibre until the atheroma grows quite large. A similar shift from dilatation to constriction of upstream proximal arteries in relation to coexisting atheroma was seen in response to the increased flow through these large arteries induced by distal intracoronary injection of non-specific dilators such as papaverine or adenosine.[136 138] This shift in the response of proximal arteries was seen also with more physiological interventions such as exercise,[139] the cold pressor test,[140] or a pacing induced increase in heart rate.[141]

Interestingly, these two last interventions, which normally increase flow through the bed, decreased flow in the presence of non-critical upstream atheromatous stenosis, suggesting that there are corresponding changes in the responsiveness of resistance vessels. Recent studies by Drexler and colleagues provide further evidence of impaired microvascular EDRF responsiveness in the human coronary arterial bed: the increase in coronary flow induced by acetylcholine relative to that induced by endothelium independent dilatation with papaverine was found to be reduced in the presence of minor coronary artery disease and/or hypercholesterolaemia[142] (and H Drexler, personal communication).

HYPERTENSION, DIABETES, SYNDROME X, HEART FAILURE, CARDIOMYOPATHY, VEIN GRAFTS

EDRF activity in large arteries can also be impaired in experimental[143–145] and clinical[146] hypertension and in diabetes.[147–149] Impaired EDRF activity may also contribute to impotence in diabetes.[150] Both hypertension and diabetes are associated with "small vessel disease" and it is notable that the experimental combination of the two conditions can cause a form of "cardiomyopathy" that seems to be the result of focal necrosis of microvascular origin.[151]

The pathogenesis of microvascular angina (syndrome X) remains unknown.[152] A relative constrictor response to ergometrine has been

described in some such patients,[153] and ergometrine is another "double agent" which both stimulates EDRF release and exerts a direct constrictor action on vascular smooth muscle.[154] It is legitimate to speculate on a possible role of impaired "feed-vessel" EDRF activity despite the limited therapeutic response to vasodilators in this condition ("lumped" pharmacological presentation of a drug may well achieve less effective dilatation than coordinated dilatation from flow stimulated release of an endogenous agent).[155]

EDRF activity in the systemic arteries has also now been reported to be impaired in heart failure[156] and in the coronary bed in congestive cardiomyopathy.[157] Conversely it has been reported to be increased by chronically elevated flow.[158] It is also impaired after preparation of vein grafts.[58]

Endothelium seems indeed to be vulnerable to many insults, with potentially far reaching pathophysiological implications.

A role in atherogenesis?

LIPOPROTEINS

Evidence is accumulating that low density lipoproteins (LDL) impair EDRF activity in vitro.[159-161] LDL can directly inactivate EDRF, but it seems likely that oxidised LDL mediates a more important and longer lasting effect. In one study, the same adverse effect on EDRF responsiveness could be induced by lysolecithin at concentrations similar to those found in the oxidised LDL: it was suggested that alteration of the composition of the endothelial lipid membrane may influence receptor function for stimulation of EDRF release.[162] LDL may be oxidised by, for example, endothelial cells, macrophages, or vascular smooth muscle cells in the arterial wall, there to be taken up by scavenger receptors on the macrophages where it accumulates to form foam cells and contribute to the atheroma.[163]

LOCALISATION OF ATHEROMA

The predilection of atheroma for certain sites implies haemodynamic influences in the process, the signal for which is thus likely to be mediated by the endothelium. The weight of evidence now points strongly to localisation of atheroma at sites of low shear stress.[164] In speculating on the possible role of EDRF in localising atheroma, two experimental observations may be relevant.

Transport of lipoproteins across vessel walls will depend on their intravascular concentration, driving pressure, wall thickness, and wall "permeability". As an example of this last determinant, Caro and Lever showed that nitrovasodilators enhanced mass transport of particles across large artery walls[165]: fewer particles accumulated on the intimal side of the media as the lattice of the medial smooth muscle was relaxed, with, by implication, faster transit across the wall. For particles, read LDL and for nitrovasodilators, read EDRF. EDRF activity is increased by high shear stress. High shear stress, via EDRF, could thus reduce the transit time of LDL across the arterial wall and the opportunity for oxidation en route. More recent work indicates that LDL moves less freely through the arterial wall than albumin, and that high transmural pressure greatly increases LDL concentrations in the intima,[166] adding further to the view that the media acts as a molecular sieve for LDL.[167] The second experimental observation derives from simple experiments in which wrapping foil around a blood vessel leads to the rapid development of atheroma.[168] One possible explanation for this is that the foil somehow prevents the egress of LDL from the adventitial surface of the vessel thereby contributing to its accumulation.

ENDOTHELIAL DAMAGE/DYSFUNCTION

Endothelial damage has long been held to be important in the atherogenic process,[169] though opponents of the hypothesis have drawn attention to the relative lack of histological evidence for endothelial damage. It is notable too that localised experimental removal of endothelium which does not damage the underlying vessel wall does not induce intimal hyperplasia.[170] Conversely, continuing minor endothelial damage as from an indwelling catheter does induce intimal hyperplasia.[171] It seems that the normal repair process after an episode of endothelial injury is self limiting, whereas prolonged stimulation of repair from recurrent endothelial damage perpetuates a chronic inflammatory response which results in the atheromatous lesion.

Endothelial cells normally divide only rarely, but they can be stimulated to divide rapidly to re-cover a denuded area. Such recently regrown cells have a somewhat different morphological appearance and their function (as manifest for example by altered EDRF responsiveness) remains abnormal for long periods. It has now been shown that other insults that fall short of causing actual denudation also result in prolonged dysfunction, associated generally with some alteration of morphological appearance. The evidence suggests that endothelium can undergo phenotypic modulation in response to several adverse stimuli, as for example the cytotoxic action of oxidised LDL. A central role of endothelium in atherogenesis might then be dependent on such phenotypic alteration, rather than on actual damage itself. Whether the altered phenotypic expression represents a single functional state characterised by both cell division and a pattern of altered function, or whether there can be variations on this theme (that is, a spectrum of altered phenotypes), remains unknown. Likewise it is not clear whether the altered state necessarily reflects the response of neighbouring endothelial cells to cryptic cell loss, or whether it can be the direct response of endothelial cells to adverse stimuli that fall short of causing actual cell death.

Reduced EDRF responsiveness is thus likely to be but one manifestation of altered endothelial function—a marker of an altered state which has other atherogenic characteristics, as for example by increasing leucocyte adhesion and attracting and promoting growth of underlying cells, leading to the atheroma which

has many characteristics of a chronic local inflammatory response.

Reduced EDRF activity itself, however, could have far reaching effects which might contribute to the atherogenic process. Nitric oxide is an oxygen radical scavenger. Less nitric oxide implies more superoxide, with greater propensity to oxidise LDL as well as contribute to further cell dysfunction. Less EDRF implies less porosity of the arterial wall and longer transit time, offering greater opportunity for oxidation en route. Less EDRF implies less inhibition of platelet adhesion with its consequent very localised release of platelet derived growth factor (which may then contribute to the cascade of events underlying intimal proliferation and atheroma). Less EDRF also means lower concentrations of cyclic GMP in the artery wall, and cyclic GMP exerts antiproliferative effects in some preparations of vascular smooth muscle cells,[172-174] possibly in relation to whether they are in the contractile or synthetic phenotype.[175]

An integrated system

A lecture such as this offers a rare opportunity for indulging in an overview of the field that seeks to discern form emerging through the mists of uncertainty which always lie ahead. Technical developments that allow us to measure and analyse flow have coincided with new conceptual insights into its control. We have moved from a cardiovascular preoccupation with pressure, faute de mieux, to an era in which we are seeing an accelerating exploration of the complexities of flow. Flow after all is what the circulation is all about. It has become clear that endothelium, sited at the interface between flowing blood and the vessel wall, plays a key role in controlling vascular structure as well as tone. Future cardiovascular physicians will surely look back on the present surge in our understanding of the vascular system as a major chapter in the evolution of our specialty of cardiovascular medicine.

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